Methyl-Branched Octanoic Acids as Substrates for Lipase-Catalyzed Reactions[1]

Philip E. Sonnet* and **Mary Welch Baillargeon**
U.S. Department of Agriculture, ARS, Eastern Regional Research Center, Philadelphia, Pennsylvania 19118

Hydrolyses of racemic methyl-branched octanoic acid thiolesters are described using six commercial lipases as catalysts. Branching at positions 2, 4 and 5 greatly reduced activity; branching at the 3-position virtually eliminated activity. The reactivities of the racemic branched thiolesters relative to the unbranched ester were very similar for each lipase preparation examined. In reactions involving configurationally pure 2-methyloctanoic acids, the S-enantiomer reacted faster both in esterification of aliphatic alcohols and in hydrolyses of aliphatic alcohol esters with all of the lipases examined. Stereobiases in hydrolyses of the octanoic acid esters branched at other positions were low and variable. In sharp contrast to the hydrolyses of the thiolesters of 2-methyloctanoic acid, two aryl esters of 2-methyloctanoic acid catalyzed by *R. miehei* lipase hydrolyzed with a bias for the R-configuration. A view of the ester-enzyme complex is offered to explain the relative rates of reaction of the racemic esters.
***Lipids* 26, 295–300 (1991).**

Triacylglycerol hydrolases [EC 3.1.1.3], more often referred to as lipases, are targets of investigation for industries that employ animal fats and vegetable oils as feedstock. A recent symposium (1) highlighted this interest and indicated wide-ranging applications for these enzymes and a desire to better understand their structure and mechanism of action.

One way to probe the active site of a lipase is with chiral fatty acids, but few instances of stereoselection using acids bearing an asymmetric center have been reported thus far (ref. 2, and references therein). We felt that a series of configurationally pure methyl branched fatty acids would prove useful for this purpose and, therefore, have synthesized a set of methyl-branched octanoic acids (3,4). An initial survey of the reactivity of six commercial lipases with selected esters of these acids is reported here. The lipases employed were crude protein preparations, and are most likely mixtures of lipolytically active species. However, since few homogeneous lipases are readily available, we selected the commercial preparations for a preliminary study. Each lipase was employed to catalyze the hydrolysis of a series of esters, and comparisons of reactivities of the branched acid esters were made with the corresponding straight chain octanoic acid ester for each lipase.

MATERIALS AND METHODS

Infrared spectra (IR) were obtained on a Perkin-Elmer 1310 spectrophotometer (Norwalk, CT) using 3% solutions in CCl_4. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained with a Bruker 300 MHz spectrometer (Karlsruhe, Germany) using $CDCl_3$ solutions with tetramethylsilane as internal standard. Gas-liquid chromatography (GLC) was done using a Shimadzu GC-Mini 2 (Columbia, MD) with an SPB-1 column (0.25 mm i.d. × 30 m) fitted with a flame ionization detector. Thin-layer chromatography (TLC) was done on silica gel 60 plates obtained from Analabs (Foxboro, MA), and fractions were visualized with iodine. Spectrophotometric assays were conducted with a Hewlett-Packard (Palo Alto, CA) 8452A Diode Array Spectrophotometer interfaced with an HP89500 Chem-Station with kinetics software (HP89512A) supplied by the manufacturer. Titrations were performed with a Radiometer Titrilab II unit (Westlake, OH).

All organic solvents were reagent grade; hexamethylphosphoric triamide (HMPT), 99%, was purchased from Aldrich Chemical Co. (Milwaukee, WI) and was stored over 13Å molecular sieves. Ellman's Reagent, 5,5′-dithio-*bis*-(2-nitrobenzoic) acid (DTNB) was similarly obtained. All other chemicals were reagent grade and were obtained from commercial sources, or, in the case of the methyl branched octanoic acids, were synthesized and characterized in this laboratory (3,4). The enzyme preparations employed in this study, their suppliers, and their specific activities in hydrolysis of triolein in 0.05 M phosphate buffer (pH 7) (μmol/min/mg) are as follows: *Candida cylindracea* (Enzeco, New York, NY) 9.45; *Rhizomucor miehei* (Gist-Brocades, Charlotte, NC) 21.3; *Rhizopus delemar* (Seikagaku Kogyo, Tokyo, Japan) 140; *Pseudomonas fluorescence* (Amano, Troy, VA) 4.3; lipoprotein lipase (Amano) 2050; and porcine pancreas lipase (Sigma Chemical Co., St. Louis, MO) 2.0. The commercial preparations were assayed for protein content using the Bradford method (5) (Bio-Rad, Richmond, CA), and bovine serum albumin was used as the standard. The percent protein for each material was as follows: *C. cylindraea*, 1.25 ± 0.12; *R. miehei*, 7.78 ± 0.74; *R. delemar*, 6.04 ± 0.58; *P. fluorescence*, 0.53 ± 0.05; liprotein lipase, 4.28 ± 4.1; and porcine pancreas lipase, 3.32 ± 0.32. The lipases were also tested for protease activity using an agar diffusion method with casein as substrate (kit from Bio-

[1]Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

*To whom correspondence should be addressed at U.S. Department of Agriculture, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

Abbreviations: DTNB, 5,5′-dithio-*bis*-(2-nitrobenzoic) acid, Ellman's reagent; E_R, enantiomeric ratio, ratio of specificity constants for enantiomers; GLC, gas-liquid chromatography; HMPT, hexamethylphosphoric triamide; IR, infrared; 2-MOA, 2-methyloctanoic acid; NMR, nuclear magnetic resonance; R and S, standard conventions for designating configuration; TLC, thin-layer chromatography.

Rad). Only the porcine pancreatic lipase showed some protease activity.

Thiolesters of 2-mercaptoethanol. Octanoic or methyl branched octanoic acid (1.40 mmol) and 2-mercaptoethanol (105 μL, 1.48 mmol) were dissolved in 5 mL of CH_2Cl_2 under dry nitrogen and cooled to 0–5°C. Dicyclohexylcarbodiimide (0.31 g, 1.47 mmol) and a few mg of *N,N*-dimethylaminopyridine were added. The resulting mixture was stirred at 25°C overnight. Hexane (15 mL) was added and the mixture was filtered into a separatory funnel; two washes of 10 mL each of hexane were added. The organic phase was shaken with 20 mL of H_2O for 3–5 min and the layers were separated. After re-extraction with another 20 mL of water, the organic phase was dried over anhydrous $MgSO_4$ and concentrated. The oily product was chromatographed over 5 g of silica gel, using mixtures of hexane and diethyl ether as solvent. Fifteen-mL fractions were collected and assayed by TLC using $CHCl_3$/acetone (90:10, v/v) as developing solvent. The thiol ester (R_f 0.33) was collected in the fractions containing 25–35% diethyl ether as eluant. The fractions showing a single spot in TLC were combined, evaporated to dryness, and finally dried *in vacuo* (0.1 mm Hg) for 1–2 hr. IR, 1680 cm^{-1}. Some of these compounds had been characterized previously as racemates (6); the new compounds also gave satisfactory chromatographic and spectral data.

p-Nitrophenyl esters. The racemic acid (0.95 g, 6.0 mmol) was dissolved in 3 mL of dry pyridine under nitrogen and cooled to 0–5°C. *p*-Nitrophenyl trifluoroacetate (1.18 g, 5.0 mmol) (7) was added and the resulting mixture was stirred without cooling for 15 min (30 min for the 2- and 3-isomers). The mixture was diluted with hexane (50 mL) and washed with 2N HCl (35 mL) and 2 × 50 mL H_2O. The organic phase was dried over anhydrous $MgSO_4$ and concentrated. The resultant oil was chromatographed on 25 g of silica gel using hexane and diethyl ether. Fractions of 100 mL each were collected, and the ester was eluted with 5–10% diethyl ether: IR, 1710, 1530, 1350 cm^{-1}; TLC (ethyl acetate/hexane, 15:85, v/v), R_f 0.40–0.44; GLC (220°C), k' = 3.93 (octanoic), 4.23–4.85 (methyl branched esters). For the octanoic acid ester: ^1H NMR, δ 0.90 (3H, CH_3, *t*,*J* = 6.8 Hz), 1.3–1.8 (10 H, *m*), 2.60 (2H, CH_2C =),*t*,*J* = 7.2 Hz), 7.28, 8.21 (4H, aryl H, *d*,*J* = 5.2 Hz) ppm; ^{13}C NMR, δ 13.9, 22.5, 24.9, 28.8, 29.1, 31.6, 34.4, 122.3, 125.1, 145.6, 155.8, 171.1 ppm.

Enzyme kinetics for hydrolysis of thiolesters. The general procedure of Renard *et al.* (8) was followed. Solutions of substrate in HMPT (up to 100 μL of stock, maintaining a volume of 100 μL with HMPT), 400 μL of DTNB in HMPT (25 mg/mL), and 2.45 mL of 0.05 M Tris buffer (pH 8) were placed in UV cells. The final solute concentrations ranged from 10^{-5} to 4 × 10^{-4}. The absorption at 412 nm (ε = 13,600 $mol^{-1}cm^{-1}$) (9) was immediately monitored for background hydrolysis (none observed) and corrected for the small amount of free thiol present in the synthesized esters. A solution of the enzyme (50 μL) was then added to initiate the reaction. For the unbranched octanoate esters it was possible to obtain a quadratic fit based on least squares of the time course data to an equation of the form: A = at + bt_2 + c. The absorbance at time t (since scanning began) is A, and a is the slope of the time trace at t = 0 and is employed as the initial rate. Kinetic parameters (K_m, V) were then obtained by a nonlinear regression curve (Marquardt analysis) (10). Many of the other substrates were much less reactive, and solubility limitations allowed only a best fit to the initial rate data to obtain a value for a pseudo first order rate constant (k_2/K_m) (Table 1). Initial velocities, v_o, were obtained with at least three initial substrate concentrations, S_o, having standard deviations ⩽ 10% using a quadratic fit to the kinetic runs. The slope of the plot of v_o *vs* S_o was divided by mg of protein used to give an apparent k_2/K_m for each substrate and each lipase preparation examined. The correlation coefficient, ϱ, for these plots was > 0.95. The values k_2/K_m for each branched acid ester relative to that of the unbranched octanoate ester for a particular lipase is given in Table 1.

Enzyme kinetics for hydrolysis of p*-nitrophenyl esters.* Solutions were prepared as described above for hydrolyzing the thiolesters, using the aryl esters and employing a molar extinction coefficient of 18,600 mol^{-1} cm^{-1} for the *p*-nitrophenate chromophore determined experimentally in this medium at this pH at 408 nm. Initial velocities for background hydrolysis of these esters (the rates were 1–2% of the enzymatic rates) were determined and then were subtracted from the rates determined in the presence of enzyme. Eight to sixteen initial substrate concentrations were used, and the time course data were then treated by nonlinear regression and fitted to a curve based on quadratic fit to obtain V_{max} and K_m. The value for V_{max} was divided by mg of protein to obtain an apparent k_2. Standard deviations for V and K_m were ⩽ 5% and 15%, respectively.

Confirmation of stereobias in kinetic studies by esterification of racemic 2-methyloctanoic acid. Reaction mixtures consisted of 2.5 mmol each of 2-methyloctanoic acid and tridecane (as an internal standard for GLC), 10 mmol of 1-butanol, and 0.25 g of enzyme powder in 10 mL of hexane saturated with H_2O. The mixures were stirred magnetically at 30°C for several days and were monitored by GLC for conversion. Analysis for configuration was performed when the conversion exceeded 20% as follows: the mixture was filtered and the clear hexane solution was extracted with cold 1 N NaOH (10 mL; to minimize racemization of the acid), and cold H_2O (5 mL). The aqueous solution was washed with diethyl ether (10 mL), and was then acidified with cold 2 N HCl (5 mL). The liberated acid was extracted into diethyl ether and converted *via* its acid chloride to an amide of S-α-phenylethylamine. Thionyl chloride (0.22 mL, 3.0 mmol) and dimethylformamide (15 μL) were added to the recovered acid in dry diethyl ether (5 mL). After standing at 25°C for 1 hr, the mixture was decanted, concentrated, dissolved in CH_2Cl_2 (5 mL), and added to a solution of S-α-phenylethylamine (0.38 mL, 3 mmol) and triethylamine (0.42 mL, 3 mmol) in CH_2Cl_2 (6 mL). After standing at 25°C for 15 min, the mixture was washed with 2N HCl, H_2O, dried over anhydrous $MgSO_4$, and concentrated. Analysis by GLC (SPB-1/210°C) gave relative amounts of the diastereomers that allowed configuration analysis of the unreacted acid. The capacity fac-

TABLE 1

k_2/K_m[a] for the Hydrolysis of Racemic Thiolesters Relative to the Octanoic Ester; Enantiomeric Ratios (Configuration of Faster Reacting Enantiomer)

		Methyl branched acid thiolesters: position of methyl group					
Enzyme source	C-8[b]	2	3	4	5	6	7
C. cylindracea	1.00[c]	0.04	0	0.09	0.05	0.47	0.45
		1.9(S)	—	4.3(S)	1.5(R)	6.2(R)	
R. miehei	1.00[c]	0.04	0.002	0.10	0.021	0.18	0.22
		2.7(S)	3.3(R)	2.0(R)	1.5(S)	1.8(R)	
R. delemar	1.00[c]	0.056	0.001	0.084	0.022	0.17	0.22
		4.6(S)	1.5(R)	1.5(R)	1	1.7(R)	
P. fluorescens	1.00[c]	0.28	0.005	0.022	0.14	0.13	0.73
		2.8(S)	9.3(R)	1.4(R)	4(R)	3.1(S)	
Porc. Pancreas	1.00[c]	0.055	0.01	0.58	0.19	0.40	1.5
		1	1	2.1(R)	3.1(R)	1.6(S)	
Lipoprotein	1.00[c]	0.001	0.003	0.008	0.11	0.16	0.92
		3.8(S)	1.9(R)	1.2(R)	6.3(R)	2.1(S)	

[a]Pseudofirst-order rate constant (min^{-1} mg^{-1}) divided by mg protein used.
[b]Octanoic acid thiolester.
[c]For each enzyme preparation: k_2/K_m from first order approximation, k_2/K_m from non-linear regression plots; *C. cylindracea:* 37.6, 45.6; *R. miehei:* 29.6, 60.5; *R. delemar:* 74.5, 146; *P. fluorescens:* 311, 173; *Porcine Pancreas:* 2.2, 1.7; *Lipoprotein:* 168, 119.

tor, k′, for the S,S-diastereomer was 3.38; and 3.66 for the R,S. Since the latter was always in excess, the S-acid reacted faster with these lipases (details in text).

Procedure for hydrolysis of racemic 2-methyloctanoic acid phenyl ester. Phenyl ester (1.0 g, 4.03 mmol) was suspended in distilled H_2O (50 mL) containing 1.0 g of NOVO "Lipozyme" which is *R. miehei* lipase from Gist-Brocades on an inert resin. The reaction mixture was agitated and kept at pH 7 using automatic titration with 0.1 N NaOH for a period of 12 hr, at which time 39.3% conversion had been achieved. The mixture was then suction filtered using CH_2Cl_2 as a rinse, and the organic phase was separated. The organic phase was washed with another portion of CH_2Cl_2, and the combined organic phase was then extracted with cold NaOH (20 mL, 1.23 N) and H_2O (10 mL). The aqueous phase was acidified with cold 2N HCl, and the product was extracted with CH_2Cl_2. The recovered acid was coverted to diastereomeric amides as above and characterized by GLC as previously described. The R,S-diastereomer predominated (72.5:27.5), which meant that the R-acid had hydrolyzed more rapidly.

RESULTS AND DISCUSSION

Octanoic acid, and the synthesized methyl branched octanoic acids, racemic as well as individual enantiomers, were converted to thiolesters of 2-mercaptoethanol using dicyclohexylcarbodiimide and catalytic amounts of N,N-dimethylaminopyridine (5). Such thiolesters can be employed in a homogeneous medium comprised of 17% hexamethylphosphoric triamide, HMPT, in aqueous buffer at pH 8 using Ellman's Reagent for a continuous spectrophotometric assay of lipase activity (8,11). Because the reaction medium is homogeneous, no information can be obtained about the particular events that mediate the special enzyme activity at an oil-water interface (12,13). On the other hand, homogeneity permits spectrophotometric analysis, gives results that are easily reproduced, and avoids ambiguities in measuring concentrations in emulsions.

The branched acid esters were much less reactive than octanoic acid ester itself and enzyme saturating conditions could not be reached without exceeding the solubilities of the substrates (ca. 5×10^{-4} M). Therefore kinetic data points for several low concentrations (10^{-5} to 10^{-4} M) were accumulated as described in Materials and Methods, and reaction velocities were calculated. At low concentrations the velocities were approximately proportional to substrate concentrations, and allowed an estimate of a pseudo-first-order rate constant (k_2/K_m) that was taken as a measure of relative activity of the substrate (Table 1). The tabulated data represent the value of (k_2/K_m) for each racemic ester relative to that of the (unbranched) octanoic ester taken as 1.00. The actual values of (k_2/K_m) obtained for the more reactive octanoic acid ester itself with each lipase, as well as the ratio that was obtained by calculating each variable independently by Marquardt analysis of nonlinear regression plots based on a wider range of substrate concentrations are footnoted. These values are dependent on enzyme purity, so the relative selectivities of a set of substrates using a single enzyme allows a better comparison of the way selectivity varies with substrate structure for a particular enzyme preparation. In addition, each enantiomer of each branched acid thiolester was also examined, and the ratios (k_2/K_m) for one enantiomer relative to the other, shown with the R,S-convention for designating configuration, are used to indicate the faster reacting enantiomer in parenthese. These values are equivalent to enantiomeric ratios, E_R, that can be obtained from kinetic resolution of racemic mixtures (14). In general, stereoselectivity was

TABLE 2

Kinetic Data for Hydrolysis of Racemic *p*-Nitrophenyl Esters

Enzyme source	Acid residue	$k_2 \times 10^3$[a]	$K_m \times 10^6$	k_2/K_m	Relative value[b]
R. miehei	Octanoic	41.8 ± 0.90	28.8 ± 3.7	1450	1.00
	2-MOA[c]	5.01 ± 0.26	47.4 ± 3.7	105	0.07
	3-MOA[c]	4.76 ± 0.13	61.2 ± 5.3	77.2	0.05
	4-MOA[c]	17.5 ± 0.39	33.0 ± 4.1	531	0.37
	5-MOA[c]	27.8 ± 0.78	78.7 ± 4.3	352	0.24
	6-MOA[c]	31.1 ± 0.78	182 ± 14	171	0.12
	7-MOA[c]	79.8 ± 3.21	121 ± 13	658	0.45
C. cylindracea	Octanoic	664 ± 21	28.3 ± 4.3	23400	1.00
	2-MOA[c]	253 ± 63	210 ± 17.8	1200	0.05
	3-MOA[c]	—	—	—	0
	4-MOA[c]	272 ± 12	77.3 ± 10.5	3650	0.16
	5-MOA[c]	87 ± 1	38.6 ± 3.9	2240	0.10
	6-MOA[c]	360 ± 12	97.7 ± 13.0	3690	0.16
	7-MOA[c]	260 ± 4	35.3 ± 3.8	7380	0.32

[a] k_2 is mol L^{-1} min^{-1} divided by mg of protein; K_m is mol/L.
[b] k_2/K_m relative to that of the unbranched ester.
[c] Methyloctanoic acid is abbreviated MOA.

TABLE 3

Kinetic Data for Hydrolysis of R- and S-2-Methyloctanoic Acid *p*-Nitrophenyl Esters

Enzyme source	Acid residue	$k_2 \times 10^3$[a]	$K_m \times 10^6$[a]	k_2/K_m	E_R[b]
R. miehei	R-2-MOA[c]	5.78 ± 0.13	40 ± 4.6	145	30.4(R)[d]
	S-MOA	0.26 ± 0.06	54 ± 6.2	4.75	
C. cylindracea	R-MOA	68.6 ± 4.8	28 ± 6.0	2650	2.2(S)[d]
	S-MOA	217 ± 11	40 ± 8.0	5830	

[a] Units as in Table 1.
[b] Ratio of k_2/K_m values for the enantiomers, also known as enantiomeric ratio.
[c] Methyloctanoic acid.
[d] Faster reacting enantiomer.

low, although the reactions of the 2-methyloctanoic acid esters were consistently selective for the S-enantiomer.

Experiments were conducted to confirm the uniform S-stereoselectivity for 2-methyloctanoic acid observed for the hydrolysis of the thiolesters. Several examples of preferential reaction of R-2-substituted acids, or acid derivatives, with lipase catalysis have been reported (2). However, in these cases the 2-substituent was always an electronegative atom, e.g., Cl, F, OC_6H_5, where nonbonded electron pairs offered electrostatic interactions that may alter stereoselection. Esterification of racemic 2-methyloctanoic acid with 1-butanol in wet hexane using lipases from *Pseudomonas fluorescence* and *Rhizomucor miehei* did indeed proceed at a faster rate for the S-2-enantiomer. For *R. miehei* at 57.0% conversion, the enantiomeric excess, ee, was 0.612, *i.e.*, the ratio of S to R in the recovered unreacted 2-methyloctanoic acid was 0.806:0.194, and therefore $E_R = 4.9$ (15). The selectivity of *P. fluorescence* was much greater and might even be synthetically useful; $E_R > 40$ (conversion: 24.8%, enantiomeric excess: 0.31). The selectivity for S-2-methyloctanoic acid, and likely 2-methyl substituted fatty acids, in hydrolysis of aliphatic esters and esterification of alcohols may be general.

The *p*-nitrophenyl esters were prepared from the trifluoroacetate of *p*-nitrophenol in pyridine (7). Lipase catalyzed hydrolysis was examined in the same medium as was used for the thiolesters, namely 17% HMPT buffered at pH 8. The maximum molar extinction coefficient for *p*-nitrophenate at that pH was determined to be 18,600 at 408 nm (at 3.33×10^{-4} M). Although the *p*-nitrophenyl esters were no more soluble than the thiolesters, their reactivity was considerably greater, and values of K_m and V were obtained using two of the lipases. *R. miehei* was chosen as a lipase characterized as positionally selective in its reactions with triglycerides, while *C. cylindracea* exemplified a lipase that is random in its action (16). The *p*-nitrophenyl esters underwent slow nonenzymatic hydrolysis at pH 8, and the kinetic data points for the enzymatic reaction were corrected by subtracting values obtained for the nonenzymatic hydrolysis rates. The background rate was 1–2% of the total rate and standard deviations for V and K_m were ≤ 6 and 15%, respectively. Table 2 gives k_2, K_m, their ratio, and the ratio relative to that of the *p*-nitrophenyl octanoate. The enantiomeric 2-methyloctanoic acid *p*-nitrophenyl esters were treated with these

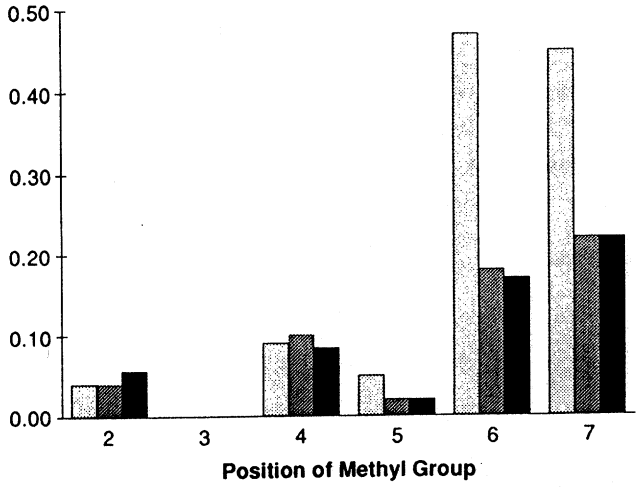

FIG. 1. Reactivity of racemic thiolesters relative to the octanoic acid ester with ⊡, *C. cylindracea;* ⊠, *R. miehei;* and ■, *R. delemar lipases.*

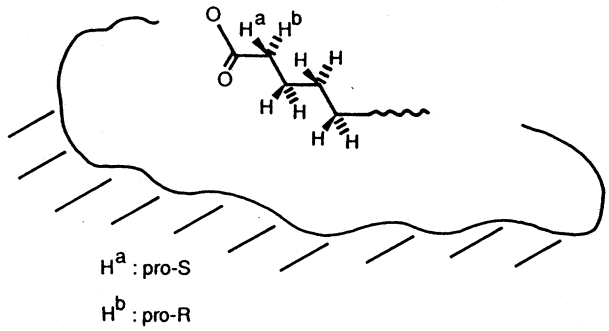

FIG. 2. Possible relationship of substrate to lipase showing carbonyl oxygen projecting into the binding pocket of the enzyme. H^a is the pro-S proton, and H^b is pro-R. The nucleophile may approach the carbonyl group from the side of H^b.

same two lipases, and the data are shown in Table 3. The S-ester hydrolyzed faster with *C. cylindracea* lipase, as had been the case for the thiolester. However, the lipase from *R. miehei* selected strongly for the R-enantiomer. This was confirmed by hydrolysis of the phenyl ester of racemic 2-methyloctanoic acid, which yielded predominantly the R-acid (E_R = 2.7). Changing the alcohol moiety from an aliphatic to an aryl alcohol had inverted stereoselection for the *R. miehei* lipase.

Although the enzymes employed were crude commercial preparations, there are features of the results that warrant discussion. Substitution at the 2-position by a methyl group markedly reduces reactivity for the esters examined. This observation has been made previously, albeit in a more limited study (17). Substitution at the 3-position generally causes a further reduction in reactivity for the lipases examined. The similarity among the lipases is quite striking, and the bar graph (Fig. 1) illustrates this using the three fungal lipases. We also noted that branching at the 5-position is often more deleterious to reactivity of the substrate than branching at positions 4 or 6. This variation in hydrolytic rate is consistent with a situation wherein the carbonyl oxygen of the ester is directed toward the enzyme in the complex (Fig. 2). Hydrophobically bound in this manner, carbons 3 and 5 of the chain would project similarly toward the enzyme, and branching at those positions would be predictably worse for binding. Branching further removed from the ester function could still affect binding but less severely; the substrate may still become hydrophobically associated with the enzyme, but use a shorter stretch of unbranched chain.

The relative rates of reaction of pairs of enantiomers of the branched acid esters are generally small. For the 2-methyl enantiomers, the K_m is similar, and the effect of the methyl branch is, perhaps predictably, on the catalytic event itself. Reaction of 2-methyloctanoic acid with these lipase preparations results in a faster reaction of the S-enantiomer when an aliphatic alcohol is involved as a nucleophile. Hydrolysis of an aryl ester, catalyzed by *R. miehei* lipase, however, displayed a reversed stereobias. This observation does not accord with a view of the enzyme binding site as a relatively static environment, but rather with one in which accommodation is possible with the stereochemical consequences reflecting the manner in which the alcohol (phenol) residue structures water in the immediate vicinity of the carbonyl group. Another possibility is the existence of a binding pocket that fortuitously accommodates an aryl ring and alters the favored direction of approach of the nucleophile to the carbonyl. The results emphasize the need for more specific protein structural information.

ACKNOWLEDGMENTS

We express our appreciation to Dr. Robert Dudley of our laboratory for obtaining NMR spectra, and to Dr. Harry Farrell for helpful discusions in the preparation of our manuscript. The lipases, excepting that of *R. delemar*, were generous gifts of the manufacturers.

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[Received May 26, 1990; Revision accepted January 29, 1991]